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Enzyme immunoassays with special reference to ELISA techniques

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SUMMARY In this review outlines are given of various types of enzyme immunoassays. The applications of such enzyme immunoassays, especially ELISA, are dealt with in detail. It is concluded that these techniques have high sensitivity and will be suitable in due course as routine laboratory tests.

During the last two decades there has been a phenomenal increase in the number and variety of immunodiagnostic tests performed. One of the reasons for this has been the development and perfection of methods which use labelled antigens or antibodies, resulting in tests with very high levels of sensitivity and specificity. Fluorescent labels have been attached to antibodies, and these conjugates are very useful for the rapid identification of organisms responsible for infectious diseases as well as for the measurement of antibody levels especially in infectious and auto-immune diseases (Nairn, 1976). Isotopes were found to be suitable, especially as labels on antigens, and radioimmunoassay (RIA) has become the method of choice for sensitive assays of both large and small molecular weight substances (Sonksen, 1974). These assays have been of especial value for steroids (Cameron *et al.*, 1975).

However, both immunofluorescence and RIA have their limitations. Immunofluorescence is tedious, time consuming, and not easily automated so it can be used for only small batches of tests. In contrast, RIA is particularly suitable for large-scale operations but the short shelf-life of the reagents, the rather sophisticated expensive equipment, and the strict regulatory controls on the use of isotopes have tended to exclude RIA from many small laboratories. These considerations have led workers to search for alternative labels for antibodies or antigens. Many candidates have been proposed for this role, for example, bacteriophages (Haimovitch *et al.*, 1970), metals (Cais *et al.*, 1977), and free radicals (Leute *et al.*, 1972) but at present the most promising new

labels are enzymes. These can be linked to antibody or antigens such that the complexes have both immunological and enzymatic activity. Degradation by the enzymes of a chromogenic or fluorogenic substrate yields an amplification factor, which enables accurate and sensitive detection of presence of the enzyme.

Most of the enzyme-immunoassays are analogous to fluorescence or radioimmunoassays in that they involve at least one separation step in which the 'bound' enzyme labelled reagent is separated from the unbound enzyme, enabling measurement of either bound or free activity. This is the basis of 'heterogeneous enzyme immunoassays', for example the 'enzyme-linked immunosorbent assay' (ELISA) which will be the main subject of this review. However, one method, the homogeneous enzyme immunoassay (Rubinstein *et al.*, 1972) deserves special mention at the outset.

Homogeneous enzyme immunoassay

A diagrammatic outline of this type of assay is given in Figure 1. Hapten is labelled with an enzyme such as lysozyme, malate dehydrogenase, or glucose phosphate dehydrogenase so that the enzyme retains its activity. However when the hapten reacts with antibody the enzyme activity of the hapten-enzyme complex is inhibited. In the test the unknown sample is mixed with the labelled hapten and with specific antibody to the hapten. If there is hapten in sample it competes with the labelled hapten for limited amount of antibody. Thus, there is less antibody available to inhibit the enzyme activity of labelled hapten. This method is the basis of

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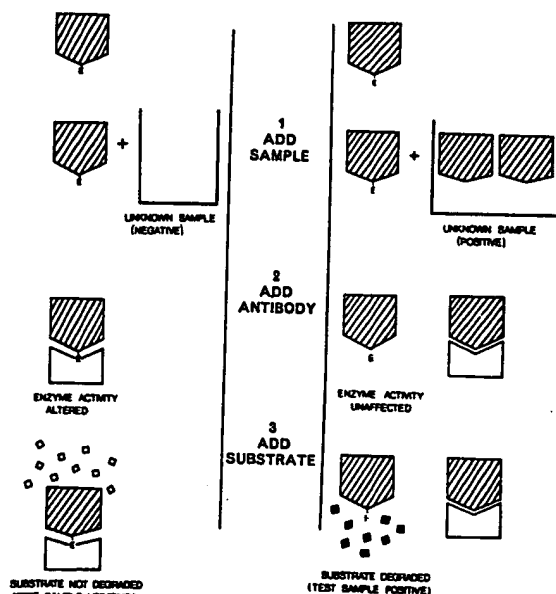


Fig. 1 The homogeneous enzyme immunoassay.

successful EMIT[®] system (Bastiani, 1978). It is especially useful for the rapid assay of low molecular weight compounds. Some representative applications of this system are listed in the Table. It is already in use on a large scale for carrying out several thousand thyroxine T₄ assays daily in laboratories in the USA (Galen *et al.*, 1976).

Table Some applications of homogeneous enzyme immunoassays

For drugs of abuse	Morphine Amphetamine Barbiturates
For cardiovascular drugs	Digoxin Propanolol Quinidine
For antidepressants	Amitriptyline Nortriptyline
For antibiotics	Vancomycin Gentamicin
Hormones	T ₃ Oestrol Cortisol

Based on material related to EMIT[®] by Bastiani (1978).

Heterogeneous enzyme immunoassay

These are known under a variety of names such as

*Syva Corporation, USA

enzyme immunoassay (EIA) (Van Weemen and Schuurs, 1971), enzyme labelled assay (ELA) (Saunders and Wilder, 1974), competitive enzyme-linked immunoassay (CELIA) (Yorde *et al.*, 1976) and enzyme-linked immunosorbent assay (ELISA) (Engvall and Perlmann, 1971). The latter is perhaps the most useful term since it identifies the heterogeneous enzyme assay yet at the same time clearly differentiates it from the tests which employ antibody-peroxidase conjugates for staining reactions for microscopy.

ELISA tests can be competitive (Fig. 2) for the assay of antigen. Enzyme labelled antigen is mixed with the test sample containing antigen, which competes for a limited amount of antibody. The reacted (bound) antigen is then separated from the free material, and its enzyme activity is estimated by addition of substrate. An alternative method for antigen measurement is the double antibody sandwich technique (Fig. 3). In this modification a solid phase is coated with specific antibody. This is then reacted with the test sample containing antigen, then enzyme labelled specific antibody is added, followed by the enzyme substrate. The 'antigen' in the test sample is 'captured' (Fig. 3-2) and immobilised on to the sensitised solid phase where it can itself then fix the enzyme labelled antibody (Fig. 3-3). This technique is analogous to the immunoradiometric assays

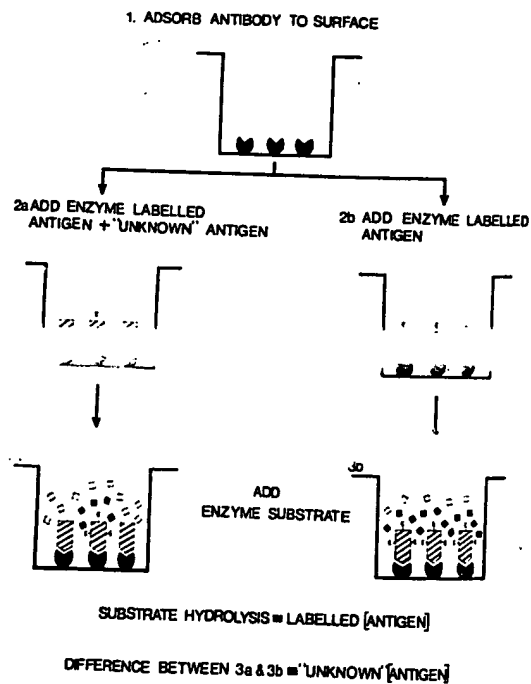


Fig. 2 Competitive ELISA for assay of antigen.

1. ANTIBODY ADSORBED TO PLATE

WASH

2. TEST SOLUTION CONTAINING ANTIGEN ADDED

WASH

3. ADD ENZYME LABELLED SPECIFIC ANTIBODY

WASH

4. ADD ENZYME SUBSTRATE

AMOUNT HYDROLYSIS = AMOUNT ANTIGEN PRESENT

Fig. 3 Double antibody sandwich method of ELISA for assay of antigen.

of Miles and Hales (1968), and has the same sensitivity and specificity for high molecular weight antigens.

ELISA has been used with enthusiasm by workers on infectious diseases, where the need is often for measurement of antibody, and the indirect method of ELISA is particularly applicable (Fig. 4) (Engvall and Perlmann, 1972).

In the indirect method the antigen is immobilised by passive adsorption on to the solid phase. Test sera are then incubated with the solid phase and any antibody in the test sera becomes attached to the antigen on the solid phase. After washing to remove unreacted serum components an antiglobulin enzyme conjugate is added and incubated. This will become attached to any antibody already fixed to the antigen. Washing again removes unreacted material and finally the enzyme substrate is added. Its colour change will be a measure of the amount of the conjugate fixed, which is itself proportional to the antibody level in the test sample.

Various other modifications of ELISA have been used, and these will be illustrated and discussed very briefly. For example, Fig. 5 shows a system where the second antibody used in the double antibody sandwich method is from a different species, and this

is then reacted with an anti-immunoglobulin-enzyme conjugate. The advantage of this is that it avoids the labelling of the specific antibody, which may be in short supply and of low potency. This same method can be used to assay antibody (in step 3) where only an impure antigen is available; the specific reactive antigens are selected by the antibody immobilised on the solid phase.

In Fig. 6 another assay for antigen is illustrated. Plates are coated with the specific antigen and the

antibody and the test sample. If there is no antigen in the test sample the reference antibody becomes fixed to

the antigen which combines with the reference antibody, which cannot then react with the sensitised solid phase. The amount of antibody attached is then indicated by an enzyme labelled antiglobulin conjugate and enzyme substrate. The amount of inhibition of substrate degradation in the test sample (as compared with the reference system) is proportional to the amount of antigen in the test system.

In all these methods passive adsorption to the solid phase can be used in the first step. Adsorption of other reagents can be prevented by inclusion of

1. ANTIGEN ADSORBED TO PLATE

WASH

2. ADD SERUM, ANY SPECIFIC ANTIBODY ATTACHES TO ANTIGEN

WASH

3. ADD ENZYME LABELLED ANTIGLOBULIN WHICH ATTACHES TO ANTIBODY

WASH

4. ADD SUBSTRATE

AMOUNT HYDROLYSED = AMOUNT ANTIBODY PRESENT

Fig. 4 The indirect method of ELISA for assay of antibody.

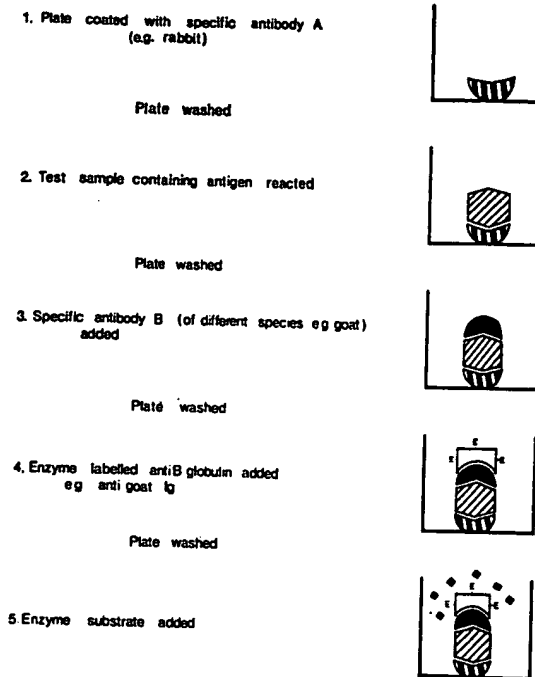


Fig. 5. The double antibody sandwich—antiglobulin ELISA for assay of antigen.

wetting agents in all the subsequent washing and incubation steps. The washing must be sufficient to prevent carry-over of reagents from one step to the next.

Aspects of methodology

SOLID PHASE

Although of little interest from an academic viewpoint the format of any diagnostic test determines whether the method is of any practical value in the laboratory. During the last few years a variety of solid phases have been used in enzyme immunoassays.

polyacrylamide with covalently bound antigen or antibody have been used (van Weemen, 1974).

washing or separation steps. The use of a solid phase such as a test tube, bead, or disc simplifies handling procedures, and antigen can be passively adsorbed to such carriers, made of polyvinyl, polypropylene (Engvall and Perlmann, 1971), polycarbonate, glass, or silicone rubber (Hamaguchi *et al.*, 1976a, 1976b). For large-scale work the disposable microtitration plates are particularly convenient (Voller *et al.*, 1974). Dipstick tests are also feasible (Felgner, 1977).

Although various carrier materials can be used, it is essential that each new type is thoroughly tested to find the amount and reproducibility of uptake of the antigen or antibody since these variables influence the results of a test (Bidwell *et al.*, 1977).

ENZYME LABELLED MATERIAL

In the ELISA method the conjugates usually consist of proteins coupled to enzymes. The coupling has been satisfactorily carried out using glutaraldehyde in either one step (Avrameas, 1969) or two steps (Avrameas and Ternyck, 1971) or by using periodate (Nakane and Kawaoi, 1974). A variety of enzymes have been used, including maleate dehydrogenase, lysozyme, and glucose 6-phosphate dehydrogenase, in the homogeneous assays, and alkaline phosphatase, horse-radish peroxidase, glucose oxidase, and β -D-galactosidase in ELISA (Avrameas, 1969; Engvall and Perlmann, 1971; Maiolini *et al.*, 1975; Kato *et al.*, 1976b). The main requirements are that the enzyme should have a high turnover number and a substrate which produces stable, soluble, easily measured products on degradation by the enzyme. The enzyme must be reasonably cheap, available in a highly purified form, and suitable for linkage with proteins.

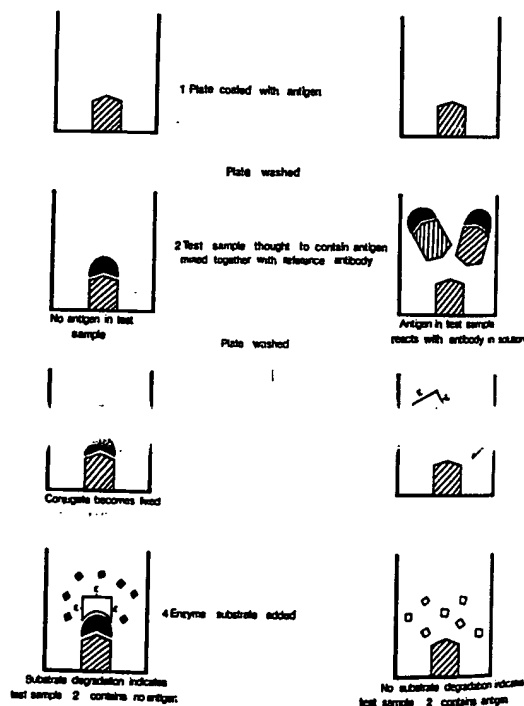


Fig. 6. Competitive antigen modification of the indirect ELISA for assay of antigen.

The one-step glutaraldehyde method has yielded satisfactory conjugates with alkaline phosphatase and horse-radish peroxidase although the ratio of enzyme to antibody varies somewhat (Avrameas, 1969). The two-step glutaraldehyde method gives conjugates in which enzyme and antibody are present in equal ratio, and this can lead to more sensitive assays (van Weemen and Schuurs, 1974). The periodate method (Nakane and Kawaoi, 1974) produces a good yield of high molecular weight con-

jugates of β -galactosidase, which have small complexes (Kato *et al.*, 1976b).

For ELISA tests an insoluble or partially insoluble product may be acceptable (for example, 5-aminosalicylic acid (5AS) or diaminobenzidine (DAB) for peroxidase conjugates). However for all quantitative ELISA tests the substrate product must be soluble. Ortho-phenylene-diamine is suitable for use with peroxidase conjugates although it is light sensitive and is reputed to have mutagenic properties. For alkaline phosphatase conjugates para-nitrophenyl phosphate is an excellent substrate. It is safe, readily available in tablet form, and gives a stable yellow product. However, peroxidase is much cheaper than alkaline phosphatase.

Fluorogenic methyl-umbelliferin substrates are highly sensitive but these are not quite so convenient for routine use.

For detailed considerations of all the above points the reader should consult the excellent review by Schuurs and van Weemen (1977).

CONDITIONS OF TEST

It is necessary to ascertain the optimum conditions for each stage of the test, for example,

- 1 optimum concentration of antibody or antigen coating of solid phase;
- 2 optimum incubation times and temperatures for sample incubation;
- 3 optimum type of substrate and time of substrate development.

These are best determined by means of a checkerboard titration using reference samples. In the case of tests for antigen, a reference sample containing a known amount of the antigen is used. For antibody tests, reference samples of positive and negative sera are employed.

RESULTS

For some purposes, especially where a yes/no result is all that is required, the results of ELISA tests can be assessed by eye. Visual reading can be made quantitative by testing a series of dilutions of the

test sample. An end-point can be established—being that dilution which produces no discernible substrate colour.

Where more precise readings are needed it is necessary to measure the substrate degradation in a spectrophotometer. For antigen assay the value of an unknown is usually read off a standard curve. For antibody assays a reference standard is incubated in the test, and the substrate reaction on that sample is allowed to proceed until it reaches a predetermined level. The reaction in other samples is stopped after the same length of time.

There is as yet no generally accepted way of making colour, or relying on optical density readings made with the spectrophotometer.

- 1 The optical density value at one dilution of the test sample. Comparison with reference +ve and -ve indicates whether or not unknown is positive.
- 2 A titre equivalent to the reciprocal of the dilution at which the test sample gives an OD the same as the reference negative sample.
- 3 The ratio of the OD of the test sample to a group of known reference negatives.
- 4 The percentile probability of the OD of the test sample being within the range of a predetermined normal (or abnormal) population.
- 5 The actual concentration of the substance being determined by reference to a standard curve prepared by plotting OD values of a series of reference samples containing different amounts of the substance.

EQUIPMENT

In its most rudimentary form ELISA can be carried out with only the sensitised plates or tubes and a series of pipettes for addition of the reagents, the results being measured visually. The next stage of sophistication is to use a spectrophotometer. This can be a relatively simple machine, the only requirements being that (a) it can be used to read optical density at the peak absorbance wavelength of the substrate, (b) it can accept microsamples (200–300 μ l), since the cost of the test is related to the volume of reagent used, and (c) readings can be carried out quickly. At present most of the spectrophotometers which are suitable accept a sample which must be transferred from the tube or plate to a microcuvette. However, one machine has been used in which the results are determined while the sample is still in the reaction tube (Leinikki and Passila, 1976). It is hoped that automatic sampling and through-the-plate readers will soon become available.

ELISA consists of a series of additions of reagents

separated by washing steps. The reagent additions can be accomplished using any of the commercially available dispensers so long as they are accurate. Special washing aspiration machines are already available for microplate ELISA. Fully automatic ELISA machines can be envisaged (Ruitenberg *et al.*, 1977) although they would be justified only by a very high work load.

COMPARISON WITH ISOTOPIC ASSAYS

It has been an article of faith that radioimmunoassays are the most sensitive and reproducible immuno assays (Landon, 1977), and some workers cling to this view in spite of the available evidence which suggests that for assays of large molecular weight substances enzyme immunoassays have the same sensitivity and precision as isotopic assays. However, for assays of small substances the radioimmunoassays are still preferable since the attachment of a label of high molecular weight, such as the enzyme, can influence the reactivity of the molecule more than the small isotope does.

There is a large fund of experience on which radioimmunoassays are based, and that background information has not yet been accumulated for enzyme immunoassays. Perhaps the main advantage of enzyme conjugates will be their stability compared with the short-lived isotopic reagent. This will make the material more readily usable by peripheral laboratories and in areas of the world where isotopes are not available. The health hazards of isotopes to the user of radioimmunoassays has perhaps been over-emphasised in the past. There is little risk in the use of ^{125}I -labelled reagents; however, the levels of radioactivity during the preparation of labelled reagents are potentially dangerous and expert handling is called for. Quite apart from the actual risks of isotopes are the legislative requirements, which can increase the costs to a laboratory, in terms of safety measures, in the use and disposal of such reagents.

At present there is a wider variety of equipment for radioassays, and although this has led to a

pretation of the results it has meant that radioimmunoassay has become an expensive tool. Enzyme immunoassays could be used in a similar way, but the level of sophistication with automation but they can be used simply and economically without sophisticated equipment.

Any suggestion that enzyme immunoassays might replace radioimmunoassays (Lancet, 1976) invariably elicits a massive response from the proponents of radioimmunoassay, who cannot envisage any alternative to the currently used methods (Ekins, 1976; Watson, 1976).

Uses of ELISA

ENDOCRINOLOGY

Initially, workers were attracted by the idea of replacing current RIA tests with ELISA. Since many of the important assays were for hormones this is the area where much of the pioneering ELISA research was done. van Weemen and Schuurs (1971, 1972, 1974, 1976) set up ELISA for measuring human chorionic gonadotrophin, and these assays were later also developed by Stimson and Sinclair (1974) and Yorke *et al.* (1976) to the point where the sensitivity approached that of RIA. Detailed evaluations of the HCG, oestrogen, and luteinising hormone assays have been made by van Weemen (1974) and van Weemen and Schuurs (1976), and all interested in this field should consult this comprehensive document. Parallel studies with ELISA on human placental lactogen (Bosch *et al.*, 1975; van Hell *et al.*, 1976; Barbour, 1976; Sadeh *et al.*, 1976) only resulted in assays which were much less sensitive than RIA. Insulin is another large hormone which has been measured successfully by ELISA (Miedema *et al.*, 1972; Ishikawa, 1973; Kato *et al.*, 1975b; Kitagawa and Aikawa, 1976). Thyroid stimulating hormone has also been assayed with accuracy at very low levels (Miyai *et al.*, 1976).

Smaller molecular weight hormones have also been measured, with a high sensitivity, including progesterone (Dray *et al.*, 1975; Gros *et al.*, 1976), oestriol (Bosch *et al.*, 1975; van Hell *et al.*, 1976; Joyce *et al.*, 1977), and cortisol (Comoglio and Celada, 1976; Ogihara *et al.*, 1977). In spite of these research communications we are not aware of any routine clinical employment of these methods at the present time.

ONCOFETAL PROTEINS

Another area where ELISA appears to offer great practical possibilities is in the measurement of onco-fetal proteins, especially in the large-scale screening for alpha-fetal protein (AFP), which is envisaged in

for AFP have been described by several groups of workers (Belanger *et al.*, 1973, 1976; Maiolini and

1976; Masseyeff *et al.*, 1976; Kirkpatrick *et al.*, 1977) and the method is in routine use in Nice, France.

Carcinoembryonic antigen levels are perhaps of less clinical importance at present but there is a growing demand for assays, and ELISA has been successfully used by Engvall and collaborators (Engvall, 1977; Engvall and Carlsson, 1976; Engvall and Perlmann, 1975; Hammarstrom *et al.*, 1975).

SERUM PROTEINS

Immunoglobulin G was first measured using competitive ELISA methods by Engvall and Perlmann (1971), Engvall *et al.* (1971), and Avrameas and Guilbert (1971). Kato *et al.* (1975a, 1976a) used the double antibody sandwich method, which together with a fluorogenic substrate resulted in an exceedingly sensitive assay.

It is rather doubtful whether enzyme-immunoassays are justified for IgG since the most sensitive

such as radial gel diffusion or the more rapid nephelometric assays have adequate sensitivity. However only RIA has previously been sensitive

developed for IgG by Honmann (1973) and by Weltman *et al.* (1976) could be of considerable practical value. Ferritin and alpha-2-haptoglobin have been assayed by ELISA but with lower sensitivity than with RIA (Miedema *et al.*, 1972; Fuccaro-Decaens *et al.*, 1974; Boenisch, 1976;) but Bartlett *et al.* (1976) obtained good correlations between ELISA and existing methods in their studies on factor VIII.

Perhaps one of the most unusual applications of ELISA in serum testing is for the detection of very small amounts of snake venom and of antibodies to the venom (Theakston *et al.*, 1977).

ANTIGENS OF INFECTIOUS AGENTS

As early as 1972, ELISA was used to measure the toxins on *Vibrio cholerae* (Holmgren and Svennerholm, 1973) but it was not followed up for bacterial antigens until recently when Carlsson *et al.* (1976) assayed antigens from brucella, yersinia, and salmonellae, and more recently by Yolken *et al.* (1977b), who detected *Escherichia coli* toxins by ELISA.

Rather more attention has been paid to viral antigen detection, and the first practical microplate ELISA system was developed by Wolters *et al.* (1976, 1977) for the detection of hepatitis B surface antigen. This was commercialised as Hepanostika (Organon Teknika), which several independent groups have found to be at least as sensitive as currently available RIA kits (Vandervelde *et al.*, 1977; Lange *et al.*, 1977; Ukkonen *et al.*, 1977). Specificity and confirmatory controls are essential, and the simple blocking procedure of Kacaki *et al.* (1977) has provided these in a convenient form.

Halbert and Anken (1977) described a rather similar double antibody sandwich assay for HBsAg, but plastic discs were used instead of microplates (Cordia H, Cordis Laboratories) as the carrier. They also reported high sensitivity together with remarkable stability and reproducibility of the reagents. Work is now progressing on ELISA for hepatitis A,

and it appears to be suitable for detection of the virus in faecal material as well as being useful for antibody assay (Duermeier *et al.*, 1977; Mathiesen *et al.*, 1977). Yolken and associates (1977a) and Scherrer and Bernard (1977) have also used ELISA for detecting viruses in stools; they were interested in the reovirus-like agents of infantile gastroenteritis. They found ELISA to be as good as electron microscopy and obviously more suitable for

There are few other reports of ELISA being used to detect viruses in clinical specimens, but Miranda *et al.* (1977) indicated that there is considerable

material. The demonstration of antigen may well become more important in other infectious diseases now that sensitive methods are available. An example of this is the work of Warren *et al.* (1977), who showed that candida antigen could prove of use in indicating invasive candidiasis. Although rather outside the scope of this journal it is of interest that ELISA has rapidly become established as the test of choice for the detection of viruses in trees and plants (Voller *et al.*, 1976a; Clark and Adams, 1977).

ANTIBODIES

The published literature indicates that the main impact of ELISA has been in the measurement of antibodies. Laboratories involved in this field often have large numbers of samples to test against many different antigens. At present a variety of procedures such as passive haemagglutination, gel diffusion, immunofluorescence, etc. are used and to a lesser extent RIA. There would be a very considerable advantage in having a single method should this prove feasible. Engvall *et al.* (1971) first showed that antibodies could be measured by indirect ELISA using enzyme-labelled antiglobulins as the indicator. To detect antibody levels in man a single enzyme-labelled anti-human immunoglobulin conjugate is suitable for most purposes, although immunoglobulin class specific conjugates such as enzyme-labelled anti-IgG, IgM, or IgA may be needed for particular purposes (Smith *et al.*, 1974; Goldblum *et al.*, 1975). Such reagents are becoming available commercially so that it is not now necessary for the user to carry out his own enzyme-antibody conjugations.

Some of the problems which may be encountered in enzyme immunoassays are illustrated by the results of tests for antibodies to DNA. Pesce *et al.* (1974) found that ELISA gave very variable results, and Engvall (1976) showed that solid phase adsorbed material, such as that used in these tests, could give erroneous results since denatured DNA is adsorbed much more than native DNA. Erikson and Ahlstedt (1977) also found in their assessment of ELISA for

diagnosis of reaginic allergy that the test was not as good at present as the isotopic assay. Lauer and Erlanger (1974) encountered no such problems when they measured antibody to adenosine.

The first large-scale uses of ELISA for antibody quantitation may well be in the area of viral diagnosis and of epidemiological viral screening, for example, to assess antibody levels after vaccination. In 1975 Voller and Bidwell (1975) showed that rubella antibody could be measured by a microplate ELISA and that the results correlated with haemagglutination inhibition; this was confirmed by Gravell *et al.* (1977). Much more extensive trials of a commercially produced kit (RUBELISA, Microbiological Associates) carried out by O'Beirne (personal communication) show that the ELISA is an efficient, sensitive, and reliable means of measuring antibody to rubella. By use of the appropriate immunoglobulin class specific conjugate it has also been possible to identify the clinically important IgM antibody response (Voller and Bidwell, 1976). Antibodies to cytomegalovirus can be assayed in a similar manner, and the ELISA may be of special use here because it needs so little antigen (Voller and Bidwell, 1976; Castellano *et al.*, 1977; Schmitz *et al.*, 1977; Anderson and Anderson, 1977; Leinikki and Passila, 1977).

The high sensitivity of ELISA has been found to be advantageous in the assay of antibody to herpes simplex virus (Gilman and Docherty, 1977), and with the appropriate antigen it even permits specific diagnosis of herpes type 2 infection (Grauballe and Vestergaard, 1977; Vestergaard and Grauballe, 1977).

Antibody levels to another member of the herpes group, Epstein Barr virus, have also been measured by ELISA (Voller *et al.*, 1976b; Wallen *et al.*, 1977). Given an antigen which can be adsorbed to a solid phase an ELISA antibody detection system could be set up for virtually any viral agent (Voller *et al.*, 1976c; Bidwell *et al.*, 1977).

Problems are more likely to arise in the interpreta-

tion of them. Leinikki and Passila (1976) have approached this problem by developing an ELISA system in which the results of test samples are com-

pared with those of reference samples. This permitted very early detection of antibodies to influenza and to mumps. Very early detection of antibodies to rabies by a sensitive ELISA was also reported by Atanasiu *et al.* (1977) and by Thraenhart and Kuwert (1977), and this could lead to a useful test for assessing vaccine responses.

Carlsson and colleagues (1972, 1975) were the first to realise that ELISA could be used in the field

of diagnostic bacteriology. They were able to quantitate antibody to Salmonella O antigens. The lipopolysaccharide antigens were immobilised quite satisfactorily by passive adsorption to the plastic carriers. The sensitivity of ELISA was at least 10-fold greater than passive haemagglutination and the Widal test, and was capable of detecting antibody in both IgG and IgM. Later Carlsson *et al.* (1976) were able clearly to differentiate the antibody response to *Brucella abortus* and *Yersinia enterocolitica* and this can be of real value since serological cross reaction between these species has made diagnosis difficult. Holmgren and Svennerholm (1973) found ELISA satisfactory for assaying antibody to *Vibrio cholerae*. They noted that immunoglobulin class specific (eg, IgG, IgM) antibodies could be measured but that there was interference between them. Jodal *et al.* (1974) were able to measure both systemic and local antibody responses to *E. coli* and their demonstration of specific antibody in urine suggested that urinary tract infections might be diagnosed by ELISA. Similar methods were also used by Russell *et al.* (1976) to assay antibodies to streptococcal M protein. There is still a large amount of treponemal serology carried out, and it could be advantageous to replace the present battery of tests by alternatives such as ELISA. Veldkamp and Visser (1975) showed that this is feasible since in their ELISA study all cases of primary and secondary syphilis were detected, as were most latent and congenital infections. Recently Halle *et al.* (1977) and Herrman *et al.* (1977) introduced ELISA for detection of antibodies against Rickettsiae and they found it suitable for both clinical and research laboratories. There is growing interest in the measurement of antibodies to mycobacteria from the diagnostic point of view. Nassau *et al.* (1976) reported on the use of ELISA for detecting antibody to *Mycobacterium tuberculosis*, and Samuels (personal communication) has adapted it for assaying antibody in leprosy patients.

There is a need for simpler methods for assessing

for whooping cough and tetanus. To date the only ELISA work has been done on tetanus antitoxin levels both competitive and the indirect methods were satisfactory (Huebner and Hoenig, 1977; Stiffler-Rosenburg and Fey, 1977; Voller *et al.*, 1977a).

The serology of parasitic diseases has attracted much attention in the last few years partly because people have become more aware of these diseases due to their importation into temperate climates and partly because the role of preventive medicine in the tropics is now appreciated. This has led to demands for diagnosis of infections in individuals and for epidemiological assessments of the diseases in

endemic areas, an application for which ELISA is ideally suited (WHO, 1976a). Malaria, still one of the world's major infections, was studied by Voller *et al.* (1974, 1975a, 1976d) in a number of geographical locations using ELISA, and the authors showed that ELISA values reflected the endemicity. Similarly, schistosomiasis can be assessed (Huldt *et al.*, 1975; Bout *et al.*, 1976; Deelder *et al.*, 1977; McLaren *et al.*, 1978). The sensitivity of ELISA is as high as

100% (Schmuck *et al.*, 1976; Voller *et al.*, 1977b). ELISA also appears to be suitable for the identification of individuals and communities in

temperate climates in developed countries is toxoplasmosis, and serology is the only practical means of diagnosis. Obviously an enzyme immunoassay could be of value here in completing the battery of TORCH investigations (toxoplasma, rubella, CMV, and herpes) on pregnant women and neonates. The toxoplasma ELISA data so far published compared favourably with the current tests such as immunofluorescence, haemagglutination, and complement fixation, and it gave very reproducible results (Bout *et al.*, 1976; Voller *et al.*, 1976c; Bullock and Walls, 1977; Walls *et al.*, 1977). ELISA has also been used for a variety of other human parasitic infections such as amoebiasis (Bos *et al.*, 1975), hydatid disease (Bout *et al.*, 1976), onchocerciasis and hookworm (Bartlett *et al.*, 1975), leishmaniasis (Hommel, 1976), and trichinosis (Engvall and Ljungstrom, 1975). The virtue of ELISA is that any department running tests can easily set it up for another disease if the appropriate antigen is made available.

Diagnosis of mycotic infections is also heavily dependent on serological tests. Hommel *et al.* (1976) showed that antibody to aspergillus and candida could be measured by ELISA, but it is perhaps of more diagnostic relevance that circulating candida antigen can also be assayed (Warren *et al.*, 1977). We are now witnessing the introduction of methods for detecting immune complexes which can be of diagnostic importance, and already ELISA has been used with success for this purpose (Alhstedt *et al.*, 1976; Casali *et al.*, 1977).

The maintenance of public health demands not only the diagnostic testing of people but also the surveillance of diseases in animals. Enzyme assays have been developed to a high level of sophistication for this purpose by Ruitenber *et al.* (1976) and by Saunders *et al.* (1977).

In this review we have attempted to survey the

method and some of the applications of enzyme immunoassays. At present few of these are available in convenient form for the clinical laboratory user but it is to be hoped that industry and public health authorities will now take the necessary measures to enable the wider use of these new tools.

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